doi:10.1038/nature22044

Supplementary Discussion

Study Rigor

a) Assurances of unbiased phenotype testing.

Consensus criteria[32] for the conduct of preclinical trials were closely followed to maintain the highest level of rigor. Mouse groups included a balance of males and females. To minimize litter effects, when groups were made for rotarod testing littermates were not housed together. All mouse treatment trials were blinded to the tester. Randomized mouse groups for rotarod tests were arranged by a one technician and rotarod testing performed by a 2nd technician not aware of group assignment of mouse genotype. Group assignments were not identifiable by the technician performing phenotype evaluations. For rotarod testing, rotarod groups were controlled for baseline rotarod performance as well as weight. When mice were transported to the neurophysiology laboratory they were encoded such that ASO treatments were blinded to the investigator performing electrophysiology testing. We also blinded the technician performing qPCR analyses using mice post phenotype testing. These measures ensured that all determinations of the effects of ASO treatments on SCA2 mice were made in an unbiased, operator-independent manner.

b) Employment of multiple phenotype testing with replication.

In our past efforts to characterize the motor phenotypes of SCA2 mice we have found the rotarod to be the most reliable test[3,4]. Rotarod tests have provided robust data with tighter standard deviations than observed in our efforts to characterize SCA2 mice using the beamwalk or DigiGait testing paradigms. We have not observed robust footprint data nor do we observe a consistent clasping phenotype for SCA2 mice. The rotarod paradigm also has the advantage that it is completely operator independent. We supplemented the behavioral phenotype with analyses of two additional phenotypes, one based on steady-state RNA and protein levels, and a second one based on neurophysiology in the acute slice. All of these were tested in two independent mouse models with independent replications. Restoration of normal or near normal Purkinje cell firing is of particular relevance as reduced PC firing is in close temporal relationship with abnormal motor behavior and like the latter declines with progression of disease.

Effect of mouse weight on rotarod test results

We determined the weights of all mice before ASO treatments and at the time of all rotarod tests. We observed no evidence that mouse weight impaired the rotarod result (Extended Data Fig. 4). For the BAC mice weight is an issue that merits further discussion because of previous findings on BAC HD mice. In one study of BAC HD mice, the increased body weight of BAC HD mice impaired rotarod performance confounding the result[33]. However unlike BAC HD mice, ATXN2-BAC-Q72 mice were lighter but performed worse than their WT littermates. At 10 weeks after ASO7 treatment, motor performance of BAC-Q72 mice had improved to a level not statistically different than the control groups (Fig. 1b). Therefore, body weight was not a confounding factor.

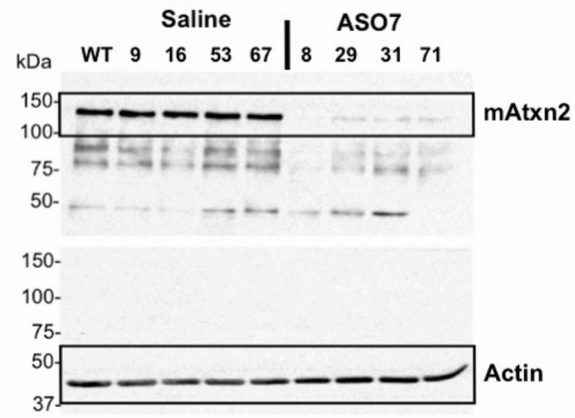
References

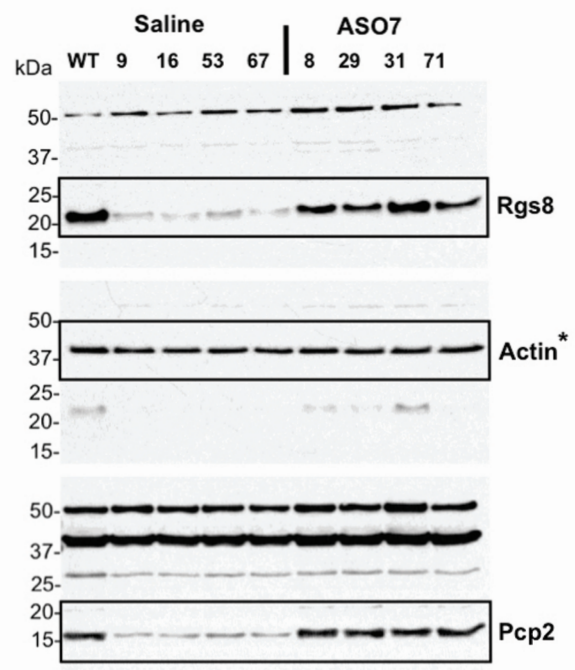
32 Landis, S. C. *et al.* A call for transparent reporting to optimize the predictive value of preclinical research. *Nature* **490**, 187-191, doi:10.1038/nature11556 (2012).

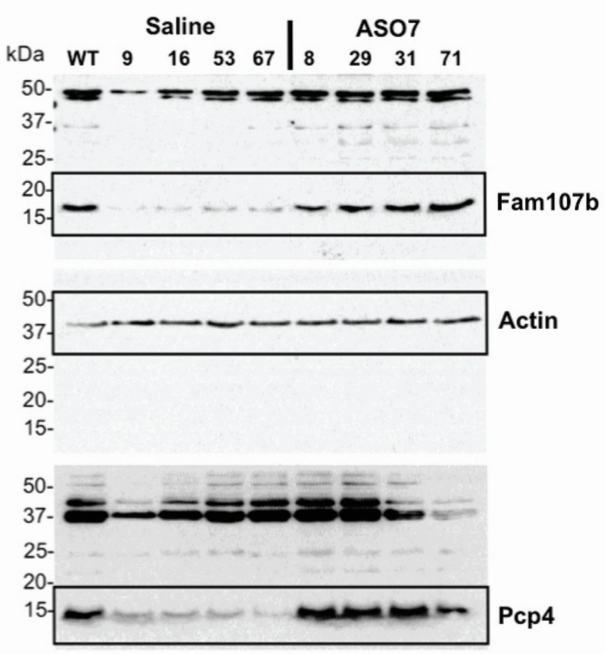
33 Kudwa, A. E. *et al.* Increased Body Weight of the BAC HD Transgenic Mouse Model of Huntington's Disease Accounts for Some but Not All of the Observed HD-like Motor Deficits. *PLoS currents* **5**, doi:10.1371/currents.hd.0ab4f3645aff523c56ecc8ccbe41a198 (2013).

Supplementary Figure 1 - Uncropped scans with size marker indications

Figure 2b







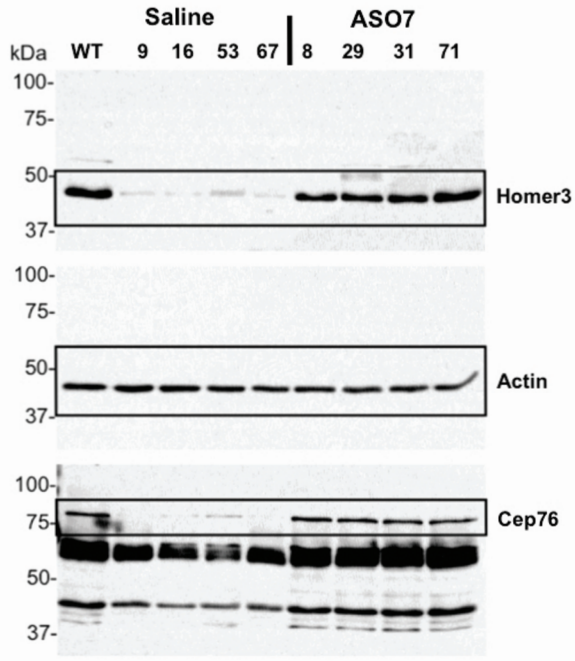
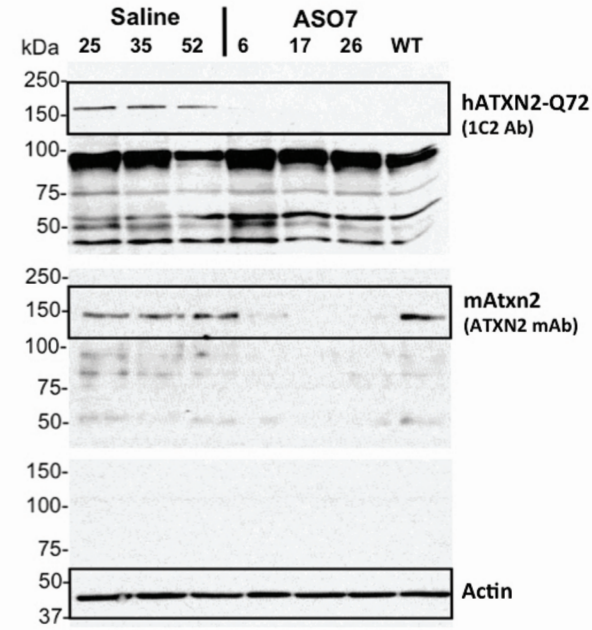
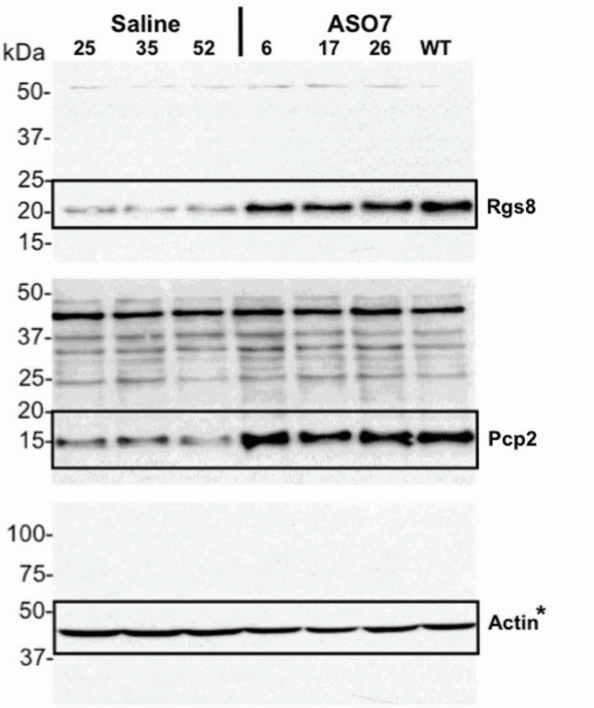
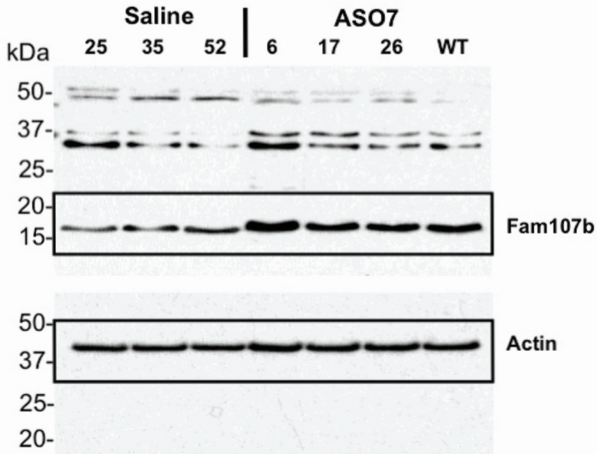
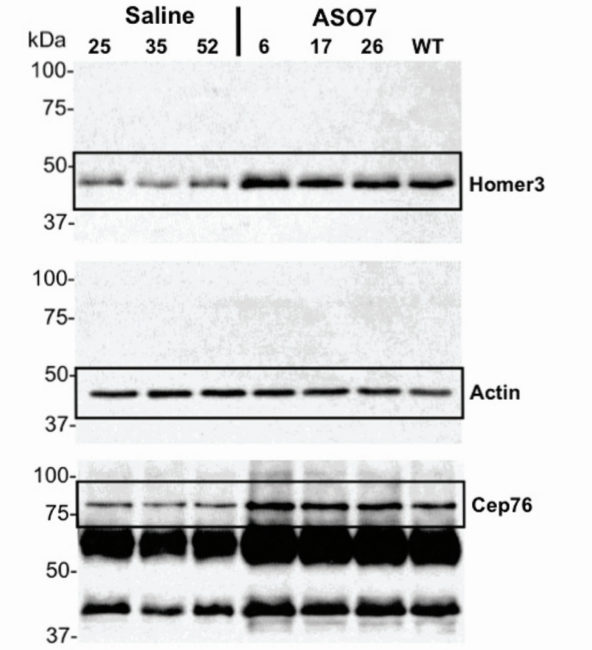


Figure 2e









***This Actin used in Figure 2**